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(54) Title: COATING OF SOLID SURFACES WITH ACTIVATED POLYHYDROXYPOLYMERS

(57) Abstract

The present invention provides for a novel, simplified method for preparing solid surfaces coated with water–soluble activated polyhydroxypolymers. The method entails contacting a solid surface which contains substantially no amino, imino, or thiol groups with a coating solution comprising an activated polyhydroxypolymer so as to obtain bonding of the activated polyhydroxypolymer to the solid surface, followed by rinsing and optionally drying of the solid surface. The contacting reaction must take place in an aqueous medium having a pH between 1.5 and 10 and or an ion strength between 0.1 and 8. Preferred polyhydroxypolymers are naturally occurring, aldehyde group–free polymers such as polysaccharides, especially dextran, but also cellulose, agarose and starch. Also synthetic polymers, especially polyvinylalcohol are preferred. The water solubility of the polymer is preferably at least 10 mg/ml and its molecular weight is preferably at least 1,000. The activation of the polymer is preferably with functional groups selected from tresyl, maleimido, cyanogenbromide, tosyl, triflyl, pentafluorobenzenesulfonyl and vinylsulphone groups. Especially preferred activated polyhydroxypolymers are tresylated, tosylated, or maleimido activated dextran. The solid surface is preferably that of an organic polymer (such as polysterene) or of glass, a ceramic or a metal. Also provided is the solid surfaces obtainable by the process as well as of the use of the surfaces in the immobilisation of biomolecules.

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COATING OF SOLID SURFACES WITH ACTIVATED POLYHYDROXYPOLYMERS

FIELD OF THE INVENTION

The present invention relates to a convenient method for coating activated polyhydroxypolymers, e.g. tresyl or maleimido activated dextran, onto solid surfaces.

BACKGROUND OF THE INVENTION

Modification of the physiochemical properties of solid surfaces by immobilising hydrophilic chemical entities thereto is known in the art. Conventional methods, e.g., include the use of hydrophobic groups or charged groups in order to facilitate the adsorption of a hydrophilic entity to a generally hydrophobic solid surface.

The present applicant's earlier international patent application, WO 94/03530, describes modification of the hydrophilic properties of solid surfaces by treatment thereof with an activated polysaccharide. In that modification process, it is required that the solid surface is carrying nucleophilic groups, e.g. amino groups or thiol group, in order to facilitate immobilisation of, e.g., periodate oxidised dextran or tresyl activated dextran thereto.

EP 596315 A2 describes a method for coating solid surfaces with dialdehyde starch comprising a contacting step and a heating step without an intermediate rinsing step. It is stated that the dialdehyde starch is nearly irreversibly attached to some polymers after the moderate heating step (50°C to 100°C). It is however also mentioned that the dialdehyde starch can be rinsed off quite easily if the heating step is omitted.

WO 91/05817 and WO 90/06954 describe the immobilisation of polysaccharides to surfaces carrying adsorbed polyamines and WO 91/09877 describes the immobilisation of, e.g., a periodate oxidised cellulose ester to a surface carrying amino groups.

WO 92/07706 describes the immobilisation of conjugates of biopolymers and polyimines to a solid surface carrying anionic groups capable of reacting with the amino groups of the polyimine.

WO 92/03732 describes the immobilisation of various water-soluble compounds onto solid surfaces, where the water-soluble compounds carry hydrophobic groups so as to facilitate the adsorption.

BRIEF DESCRIPTION OF THE INVENTION

It has now surprisingly been found that coating of solid surfaces with polyhydroxypolymers can be accomplished by very simple means, i.e. without a mandatory prior activation of the surfaces to be coated and without inclusion of, e.g., aldehyde groups, amino group or hydrophobic group in the polyhydroxypolymer. It has in particular been found that the coating of microtitre plates (e.g. polystyrene microtitre plates) with activated polyhydroxypolymers (e.g. activated polysaccharides such as tresyl activated dextran (TAD) or maleimido activated dextran (MAD)) can be accomplished without the need for prior coating of the surface with a polyamine or a polyimine and even without prior conjugation of the polyhydroxypolymer with polyimines, polyamines, hydrophobic ligands, and the like.

Thus, the present invention provides a method for coating a solid surface with a water-soluble activated polyhydroxypolymer, where the solid surface comprises substantially no amino groups, imino groups or thiol groups, the method comprising the step of:

a) contacting a coating solution of the activated polyhydroxypolymer in an aqueous medium having a pH in the range of 1.5-10 and/or having ion strength in the range of 0.1 to 8 with the solid surface so as to obtain a bonding of the activated polyhydroxypolymer to the solid surface;
b) rinsing the solid surface having the activated polyhydroxypolymer attached thereto with a rinse solution; and
c) optionally drying the solid surface having the activated polyhydroxypolymer attached thereto.

The present invention also relates to post-treatment of the coated surface so as to convert the functional groups to other functional groups. Furthermore, the present invention

relates to the solid surfaces obtained, their use in immobilisation of biomolecules, as well as the thus obtained solid surfaces comprising immobilised biomolecules.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1

Binding of a peptide to polystyrene microtitre wells coated with TAD at different pH values. The wells coated with TAD at acidic pH bind more peptide than wells coated at basic pH. It is not clear if this effect is due to improved adsorption of the TAD at low pH or due to hydrolysis of the tresyl groups at high pH.

Figure 2

Chemical specificity of the TAD coated surface. The amino acids which were protected on the α-amino group could bind to the tresyl groups with the side chain only. The TAD surface showed pronounced preference for lysine and cysteine, i.e. the chemical specificity was restricted to amine and thiol.

Figure 3

A B-cell epitope scan using overlapping peptides performed on a TAD coated microtitre plate (A) and a conventional microtitre plate (B, MAXISorp). After immobilizing the peptides (derived from tumor necrosis factor α (TNFα)) on the two different plate types, TNFα antiserum was added. Peptides containing B-cell epitopes were then expected to be recognized by the antiserum. It is obvious that more peptides were recognized on the TAD coated surface than on the conventional plate.

Figure 4

TAD coated on surfaces other than organic polymers. A glass test tube and a nickel spatula were treated with TAD as described in Experimental and tested for ability to bind the peptide biotin-MP9. The controls (- TAD coating) were a glass test tube and a nickel spatula respectively, treated with the coating solvent only. For comparison an OD_{490}/cm^2 value was calculated. Obviously, only the TAD coated surfaces bind the peptide.

Figure 5

The effect of NaCl when coating MAD on a polystyrene microtitre plate (MAXISorp). The presence of NaCl increased the binding of cysteine by approx. 25%. The presence of cysteine binding was detected by adding biotin-NHS. The signal from the lysine as well as the signal from the control with buffer only was very low. This documents that the binding to the MAD surface is thiol specific.

Figure 6

Coating a polystyrene microtitre plate (MAXISorp) with different concentrations of MAD. The optimal coating concentration is between 0.25 and 1 mg/ml. Binding of cysteine decreased with decreasing concentration of MAD. The binding of lysine at all MAD concentration was very low.

Figure 7

Chemical specificity of the MAD coated surface. The amino acids which were protected on the α-amino group could bind to the maleimido groups with the side chain only. The MAD surface showed pronounced preference for cysteine, i.e. the chemical specificity was restricted to thiol.

Figure 8

Generation of a surface with functional carboxylic acid groups. A TAD surface prepared as described in Example 1 was reacted with 6-amino hexanoic acid under various conditions. The activation with EDC and NHS provided a specific conversion of the tresyl functional groups to carboxylic acid functional groups.

Figure 9

Generation of a surface with functional thiol acid groups. A TAD surface prepared as described in Example 1 was reacted with 2,2'dithio-bis(ethylamine). Subsequent reaction of the TAD surface and the thiol-modified TAD surface with biotin-maleimide and biotin-MP9 showed that the TAD surface was specific for amines and the thiol-modified surface was specific for maleimide without any significant cross-specificity.

DETAILED DESCRIPTION OF THE INVENTION

As described above, the present invention relates to a method for coating surfaces with activated polyhydroxypolymers such as activated polysaccharides.

(The polymer)

Polyhydroxypolymers include naturally occurring polyhydroxy compounds such as polysaccharides and synthetic polyhydroxy compounds such as synthetic organic polymers e.g. polyvinylalcohol and poly(hydroxymethylmethacrylate). An important common feature of such compounds is that they are relatively hydrophilic, which is reflected in a good water solubility.

Illustrative examples of naturally occurring polyhydroxy compounds are polysaccharides, gum xanthan, etc. Illustrative examples of synthetic organic polymers are polyvinylalcohol, poly(hydroxymethylmethacrylate), poly(hydroxyethylmethacrylate), poly(hydroxypropylmethacrylate), etc. as well as the corresponding copolymers.

The term "polysaccharide" is intended to be used with its normal meaning, i.e. "a combination of nine or more monosaccharides, linked together by glycosidic bonds", cf. Hawley's Condensed Chemical Dictionary, 11th ed., Sax and Lewis, eds., Van Nostrand Reinhold Co., New York, 1987. Examples of such polysaccharides are dextran (e.g. Dextran 40, Dextran 70, Dextran 75), agarose, cellulose and starch.

The present invention is considered especially applicable for polysaccharides and polyvinylalcohol, in particular polysaccharides such as dextran.

The (weight) average molecular weight of the native polyhydroxypolymer in question (i.e. before activation) is typically at least 1,000, such as at least 2,000, preferably in the range of 2,500-2,000,000, more preferably in the range of 3,000-1,000,000, in particular in the range of 5,000-500,000. It has been shown in the examples that polyhydroxypolymers having an average molecular weight in the range of 10,000-200,000 are particularly advantageous.

It is important that the solubility of the polyhydroxypolymer is so that solvation of the polymer is preferred over non-specific adsorption to the solid surface. Thus, In order to exploit the full scope of the present invention, the polyhydroxypolymer is preferably wa-

ter soluble to an extent of at least 10 mg/ml, preferably at least 25 mg/ml, such as at least 50 mg/ml, in particular at least 100 mg/ml, such as at least 150 mg/ml. It is known that dextran, even when activated as described herein, fulfils the requirements with respect to water solubility.

For some of the most interesting polyhydroxypolymers, the ratio between C (carbon atoms) and OH groups (hydroxy groups) of the unactivated polyhydroxypolymers (i.e. the native polyhydroxypolymer before activation) is in the range of 1.3 to 2.5, such as 1.5-2.3, preferably 1.6-2.1, in particular 1.85-2.05. Without being bound to any specific theory, it is believed that such as a C/OH ratio of the unactivated polyhydroxypolymer represents a highly advantageous level of hydrophilicity. Polyvinylalcohol and polysaccharides are examples of polyhydroxypolymers which fulfil this requirement. It is believed that the above-mentioned ratio should be roughly the same for the activated polyhydroxypolymer as the activation ratio should be rather low.

The term "native polyhydroxypolymer" and similar terms are intended to mean the polyhydroxypolymer before chemical modification. Thus, in a native polysaccharide substantially all monosaccharide units are intact and recognisable.

As mentioned above, the polyhydroxypolymers carry functional groups (activation groups), which facilitates the anchoring of secondary molecules (e.g. peptides, proteins, antibodies, antigens, nucleic acids, etc. (see below)) to the solid surface. A wide range of applicable functional groups are known in the art, e.g. tresyl (trifluoroethylsulphonyl), maleimido, cyanogenbromide, tosyl (p-toluenesulfonyl), triflyl (trifluoromethanesulfonyl), pentafluorobenzenesulfonyl, and vinyl sulphone groups. Preferred examples of functional groups within the present invention are tresyl, maleimido, tosyl, triflyl, pentafluorobenzenesulfonyl, and vinylsulphone groups, among which tresyl, maleimido, and tosyl groups are particularly relevant.

It is believed to be advantageous that the functional groups of the activated polyhydroxypolymers according to the invention are attached to the polyhydroxypolymer via a fraction of the hydroxy groups of the native polyhydroxypolymer. Thus, it is preferred that the skeleton of the native polyhydroxypolymer is substantially unaffected by the activation.

Hence, it is also believed that aldehyde functionalities, e.g. arising from periodate oxidation of polysaccharides, may be disadvantageous as functional groups of the activated polyhydroxypolymer as oxidation of a diol to two aldehyde groups markedly reduces the hydrophilicity of the polyhydroxypolymer. Thus, preferably, substantially no aldehyde groups are included in the polyhydroxypolymer other than any (normally masked) aldehyde functionalities of a native polysaccharide. Functional groups should in particular not be aldehyde groups arising from treatment of a polysaccharide with excessive amounts (i.e. more than 1 mole per mole hydroxy groups in the polysaccharide) of periodate.

Furthermore, for the reason mentioned before, it is also preferred that other functional groups are not attached to the polyhydroxypolymer via carbon atoms arising from oxidation of diols to two aldehydes. Thus, it is preferred that a polysaccharide used within the scope of the present invention is substantially unmodified before activation with functional groups.

It should be understood from the above, that the functional groups (activation groups) are not polymers in themselves as the method according to the invention is simpler as known methods where e.g. poly-L-lysine and other (poly)amines/(poly)imines are used as "activation groups" for immobilising a polysaccharide to a solid surface. It should in particular be understood that the functional groups are not polyimines such as polyethylene imine or polyamines such as poly-L-lysine. Thus, preferably substantially no amino (primary, secondary and tertiary aliphatic and aromatic amines), imino, ammonium (aliphatic and aromatic ammonium groups such as pyridinium groups), and thiol groups should be included in the polyhydroxypolymers when used within the present invention. It should also be understood that hydrophobic ligands (e.g. phenyl, naphthyl, pyridyl and pyridone groups) which may facilitate the immobilisation of a polysaccharide to some solid surfaces in conventional method are not to be construed as "activation groups" within of the present invention, and that substantially no groups of this character should be included in the polyhydroxypolymer.

It is however believed that the functional groups are some-how involved in the adsorption of the polyhydroxypolymer to the solid surface. This is i.a. illustrated by the difference in the optimal conditions for coating of dextran carrying different functional groups (tresyl and maleimido).

The polyhydroxypolymers are generally prepared by methods known to the person skilled in the art.

Tresyl activated polyhydroxypolymers can be prepared using tresyl chloride as described for activation of dextran in Example 1 or as described in Gregorius et al., J. Immunol. Meth. 181 (1995) 65-73.

Maleimido activated polyhydroxypolymers can be prepared using *p*-maleimidophenyl isocyanate as described for activation of dextran in Example 3. Alternatively, maleimido groups could be introduced to a polyhydroxypolymer, such as dextran, by derivatisation of a tresyl activated polyhydroxypolymer (such as tresyl activated dextran (TAD)) with a diamine compound (generally $H_2N\text{-}C_nH_{2n}\text{-}NH_2$, where n is 1-20, preferably 1-8), e.g. 1,3-diaminopropane, in excess and subsequently react the amino groups introduced in TAD with reagents such as succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), sulfo-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), succinimidyl 4-(*p*-maleimidophenyl)butyrate (SMPB), sulfo-succinimidyl 4-(*p*-maleimidophenyl)butyrate (sulfo-SMPB), N-γ-maleimidobutyryloxy-succinimide ester (GMBS) or N-γ-maleimidobutyryloxy-sulfosuccinimide ester. Although the different reagents and routes for activation formally results in slightly different maleimide activated products with respect to the linkage between the maleimide functionality and the remainder of the parent hydroxy group on which activation is performed, all and every are considered as "maleimide activated polyhydroxypolymers".

Tosyl activated polyhydroxypolymers can be prepared using tosyl chloride as described for activation of dextran in Example 2. Triflyl and pentafluorobenzenesulfonyl activated polyhydroxypolymers are prepared as the tosyl or tresyl activated analogues, e.g. by using the corresponding acid chlorides.

Cyanogenbromide activated polyhydroxypolymer can be prepared by reacting the polyhydroxypolymer with cyanogenbromide using conventional methods. The resulting functional groups are normally cyanate esters with two hydroxy groups of the polyhydroxypolymer.

The degree of activation can be expressed as the ratio between the free hydroxy groups and the activation groups (i.e. functionalised hydroxy groups). It is believed that a ratio

between the free hydroxy groups of the polyhydroxypolymer and the activation groups should be between 250:1 and 4:1 in order to obtain an advantageous balance between the hydrophilicity and the reactivity of the polyhydroxypolymer. Preferably the ratio is between 100:1 and 6:1, more preferably between 60:1 and 8:1, in particular between 40:1 and 10:1.

Especially interesting activated polyhydroxypolymers for use in the method according to the invention are tresyl, tosyl and maleimido activated polysaccharides, especially tresyl activated dextran (TAD), tosyl activated dextran (TosAD), and maleimido activated dextran (MAD).

(The solid surface)

The solid surface to which the polyhydroxypolymer is attached can be selected from a wide variety of solid surfaces used in the analytical and diagnostic fields, however the solid surfaces are generally characterised in the lack of chemical functionalities (e.g. amines, imines and thiols) which are believed to facilitate the coating of surfaces with activated polyhydroxypolymers in conventional methods. The most important types of solid surfaces are those of organic polymers, glasses, ceramics and metals.

Among the organic polymers, polystyrene, polycarbonate, polypropylene, polyethylene, polyethyleneglycol terephthalate, polyvinylacetate, polymethylpentene, polyvinylpyrrolidinone, polyacrylonitrile, polymethylmethacrylate and polyvinylchloride are illustrative examples, where polystyrene and polycarbonate are especially interesting examples.

Among the glasses and ceramics, borosilicate glass (Pyrex glass) and soda-lime glass are especially relevant examples, e.g. in the form of specimen tubes, vials, and slides for microscopy. The surface of the glass may be treated with acid prior to coating.

Among the metal, nickel, iron, copper gold, silver, aluminium and zinc are the most relevant illustrative examples. Such surfaces are normally cleaned before coating in order to remove any metal oxides.

Preferably the solid surface is the surface of a polystyrene body, a polycarbonate body, a borosilicate glass body, or a soda-lime glass body.

The body in itself may have a form or may be designed and shaped for the particular desired use. E.g. the body may be in the form of a sheet, a film, a bead, a pellet, a disc, a plate, a ring, a rod, a net, a filter, a tray, a microtitre plate, a stick, or a multi-bladed stick. Especially interesting bodies to be coated according to the present invention are microtitre plates, e.g. polystyrene microtitre plates, sticks and beads.

It is understood that the surface of the body in question is not already chemically modified by coating with a compound before use in the present method. In particular, the surface of the body is not carrying amino, imino or thiol groups. The surface may however be irradiated so as to modify the chemical and/or physical properties of the surface (typically an oxidative process). It has been shown that irradiation is irrelevant in one case (tresyl activated dextran) and slightly advantageous in another case (maleimido activated dextran).

Particularly interesting examples of solid surfaces are the surfaces of polystyrene microtitre plates, polystyrene beads, polystyrene sticks, polycarbonate microtitre plates, glass beads, and glass plates.

(The method)

As mentioned above, the method according to the present invention includes a number of steps, i.e. the contacting step, the rinse step, and the optional drying step. These steps will be described in detail in the following:

a) contacting step

As the activated polyhydroxypolymers are inherently water soluble, the coating solution comprising the activated polyhydroxypolymers is preferably an aqueous solution. Apart from the polyhydroxy polymers, such an aqueous solution comprises a pH adjusting agent and/or a chaotropic agent, and optional one or more auxiliary components.

Water is preferred as the solvent i.a. for environmental reasons, economic reasons and because many organic polymer materials, such as polystyrene, are damaged by various organic solvents such as dimethyl formamide and acetone. Furthermore, organic solvents

often interfere with physical, non-covalent adsorption to solid phases. It is thus preferred that the solvent comprises less than 5% of organic solvents constituents, more preferably no organic solvent constituents are included.

The concentration of activated polyhydroxypolymer in the coating solution is typically in the range of 0.001 mg/ml to 5 mg/ml, typically in the range of 0.01 mg/ml to 1 mg/ml, and preferably in the range of 0.1 mg/ml to 0.5 mg/ml.

With respect to the required amount of activated polyhydroxypolymer per area unit (area of the surface to be treated), it is believed that in the range of 0.01-500 $\mu g/cm^2$ will be suitable in order to obtain a uniform coating, preferably in the range of 0.06-200 $\mu g/cm^2$, in particular in the range of 0.1-50 $\mu g/cm^2$, is used.

As mentioned above, the coating solution which is contacted with the solid surface has a pH in the range of 1.5-10 and/or a ion strength in the range of 0.1 to 8.

The desired pH value is obtained by using a pH adjusting agent in the solution of the activated polyhydroxypolymer. pH adjusting agents may be acetic acid, (e.g. 0.5% acetic acid pH 2.6), a citrate/phosphate buffer (e.g. 0.035 M citrate, 0.075 M phosphate, pH 5.0), phosphate buffered saline (PBS) (e.g. 0.01 M phosphate, 0.15 M NaCl, pH 7.2) or a carbonate buffer (e.g. 0.1 M carbonate, pH 9.6). When an acidic pH is desirable, other simple organic acids, e.g. formic acid, propionic acid, and butanoic acid, in concentrations in the range of 0.1-10%, trifluoroacetic acid (0.05-5%), trichloroacetic acid (0.05-5%), HCl (0.01-1 M), H_2SO_4 (0.01-1M) may also be used. For pH adjustment of the coating solution acetic acid and HCl are very convenient as "left over" will be removed by evaporation be evaporation in the drying process.

Thus, the pH of the coating solution is typically in the range of 1.5-10, preferably in the range of 2.0-7.5, more preferably in the range of 2.0-5.5. It has been shown for a tresyl activated polyhydroxypolymer that equally advantageous products are obtained within the pH range of 2.0-5.5.

In the case of coating a solid surface with tresyl activated dextran, it is clear from Fig. 1 that low pH is preferred. Coating at e.g. pH 8 might also work if the coating time is reduced in order to minimise hydrolysis of the tresyl groups. However, in a large scale pro-

duction it is inconvenient to have a very narrow time frame, and as tresyl activated dextran seems to be very stable at e.g. 0.5% acetic acid in water (approx. pH 2.6) this is a very suitable solvent composition.

The desired ionic strength is obtained by using a chaotropic agent in the solution of the activated polyhydroxypolymer. The chaotropic agent must not contain groups (e.g. thiols or amines) that react with the reactive sites of the activated polyhydroxypolymer or have any adverse effect on adsorption of the activated polyhydroxypolymer to the solid phase (as can e.g. be experienced with some detergents). NaCl, from 0.5 M to 4 M in water has been used with satisfactory result. Alternatively, guanidinium chloride, sodium thiosulphate, and sodium thiocyanate may be used. Lower concentration can be used but there is a tendency to increased variability of the coating with decreasing concentration of the chaotropic agent. Thus, the ionic strength of the solution of the polyhydroxypolymer is typically 0.1-8, preferably 0.5-6, more preferably 0.8-5, in particular 1.2-4.

It should be understood that a pH adjusting agent may be used in conjunction with a chaotropic agent so as to fulfil both the pH and the ionic strength recommendations.

Auxiliary agents may also be included in the coating solution, however, preferably no other constituents are included as it is preferred that all ingredients are efficiently removed at least in the drying step.

The coating time can be from 1 min to over night, e.g. from 3 hours to over night, but the coating time appears to be uncritical. In large scale production over night coating is often very convenient.

The coating can be performed at temperature ranging from 4°C to 56°C with equally good results. However, room temperature is very convenient in order to reduce temperature caused edge effects in e.g. microtitre plates which otherwise could lead to variability in performance between a well in the centre on the microtitre plate and a well close to the edge of the microtitre plate. Furthermore, evaporation during incubation is less or virtually absent at room temperature compared to elevated temperature.

Preferred conditions are coating over night at room temperature. These conditions have proven advantageous for tresyl activated dextran (TAD) as well as for maleimido activated dextran (MAD).

After the prescribed coating time, the coating solution is removed from the solid surface or the solid surface is removed from the coating solution, whatever is most convenient. In the case of a microtitre plate, the coating solution is normally decanted or pipetted off. It should be understood that the coating solution is normally not allowed to completely evaporate, as the performance of the coating is thereby beyond control in that a fraction of the polyhydroxypolymer will be attached to the solid surface by weak passive adsorption and may not be efficiently rinsed off in the subsequent step.

b) rinse step

The rinse solution is typically an aqueous solution or simply water. When acidic conditions are applied in the contacting step, an acidic solution is advantageously used in the rinse step in order to avoid that unspecific binding of remaining polyhydroxypolymer or other components takes place due to a change in pH. The rinse solution should contain no components that could react with the reactive sites of the activated polyhydroxypolymer (e.g. amino or thiol groups) or interfere with the coating and should be easy to remove in the drying process. Preferably, the rinse solution contains only little salts or other non-volatile components.

Acetic acid, e.g. 0.5% in water, is very suitable as the rinsing solution in situation where acidic conditions have been used in the contacting step as it has a low pH value (approx. 2.6) and is easily removed by evaporation in the drying process. Water is suitably used where the contacting step has been performed under chaotropic conditions.

c) drying conditions

After the rinse step, the surface is dried in order to remove the rinsing solution and other volatile components, e.g. acetic acid. Drying is important especially for storage of the coated surface and can be performed at a temperature in the range 20°C-56°C, preferably in the range of 20°C-45°C, with good results. Drying at around 37°C ensures that a relatively fast evaporation of residual rinsing solution takes place and is often more ad-

vantageous for large scale production of e.g. coated microtitre plates than drying at 56°C. The drying time can be further reduced under reduced pressure.

After drying, the coated surface can be stored for later use, or may be used shortly after. When the coated surface is used immediately after preparation, the drying step may even be omitted if the rinse solution is compatible with the solutions used in the desired application.

It should be noted that the coated surfaces prepared according to the present invention have an excellent storage stability expressed as a shelf-life of more than 2-3 years.

Thus, it is preferred and also realistic within the present invention that the stability of the coated surface is so that the difference in absorbance for the most absorbing amino acid in a test for the amino acid side chain specificity (as described for TAD in example 6) when tested on an uncoated solid surface and on a similar solid surface coated with the activated polyhydroxypolymer in question has decreased with at the most 25%, preferably at the most 15%, more preferably a the most 10%, in particular at the most 5%, after storage at 37°C for one year. Storage is effected at ambient conditions with respect to atmospheric pressure and atmospheric composition. It is believed that especially preferred coated surfaces obtained according to the method according to the invention fulfil these requirements even when stored at 50°C for one year under the same conditions.

In a preferred embodiment of the present invention the polyhydroxypolymer is a polysaccharide, in particular dextran. Especially important functional groups in connection with polysaccharides (e.g. dextran) are tresyl, tosyl and maleimido,

Thus, in one preferred embodiment of the present invention, the method comprises

a) contacting a solution of a tresyl activated polysaccharide in an aqueous medium having a pH in the range of 1.5-7.5 with a polystyrene surface;
b) rinsing the polystyrene surface with a rinse solution; and
c) drying the polystyrene surface coated with the tresyl activated polysaccharide.

In another preferred embodiment of the present invention, the method comprises

a) contacting a solution of a maleimido activated polysaccharide in an aqueous medium having a ionic strength in the range of 0.5-6 with a polystyrene surface;

b) rinsing the polystyrene surface with a rinse solution; and

c) drying the polystyrene surface coated with the maleimido activated polysaccharide.

In still another preferred embodiment of the present invention, the method comprises

a) contacting a solution of a tosyl activated polysaccharide in an aqueous medium having a pH in the range of 1.5-7.5 with a polystyrene surface;

b) rinsing the polystyrene surface with a rinse solution; and

c) drying the polystyrene surface coated with the tosyl activated polysaccharide.

For the above-mentioned preferred embodiments, the requirements and recommendations mentioned above with respect to the solid surface, the coating conditions and the polyhydroxypolymer also apply.

(Uses)

The coated solid surfaces carrying an activated polyhydroxypolymer may be further functionalised before their final use or some or all of the functional groups of the activated polyhydroxypolymer may be reacted so as to form other functional groups. In this way, functional groups with the ability to facilitate the coating of a solid surface with a polyhydroxypolymer may be chosen in the initial process (step a) to c)) and these functional groups may afterwards be converted to other functional groups. The tresyl and tosyl groups are good examples of such functional groups for the initial process.

As an example, a tresyl activated polyhydroxypolymer may be reacted with an ω-amino carboxylic acid (generally $H_2N\text{-}C_nH_{2n}\text{-}COOH$, where n is 1-20, preferably 1-8) so as to form an immobilised carboxylic acid functionalised polyhydroxypolymer. This is illustrated in Example 10.

In another variant, a tresyl activated polyhydroxypolymer may be reacted with an α,ω-diamino-alkane (generally $H_2N\text{-}C_nH_{2n}\text{-}NH_2$, where n is 1-20, preferably 1-8) so as to form an immobilised amino functionalised polyhydroxypolymer.

In still another variant, a tresyl activated polyhydroxypolymer may be reacted with a cystamine or an analogue (generally $H_2N\text{-}C_nH_{2n}\text{-}S\text{-}S\text{-}C_nH_{2n}\text{-}NH_2$, where n is 1-10, prefer-

ably 1-4) and subsequently reduced (e.g. with sodium dithionite) so as to form an immobilised thiol functionalised polyhydroxypolymer. This is illustrated in Example 11.

Furthermore, the formation of the maleimido functionalised polyhydroxypolymer via the tosyl activated polyhydroxypolymer described above may also be accomplished after coating of the solid surface by reacting an immobilised amino functional polyhydroxypolymer with a maleimide reagent, e.g. a reagent selected from succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (SMCC), sulfo-succinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC), succinimidyl 4-(*p*-maleimidophenyl)butyrate (SMPB), sulfo-succinimidyl 4-(*p*-maleimidophenyl)butyrate (sulfo-SMPB), N-γ-maleimidobutyryloxy-succinimide ester (GMBS) and N-γ-maleimidobutyryloxy-sulfo-succinimide ester.

The above-mentioned variants also apply for other functional groups (e.g. tosyl, triflyl, the cyanogenbromide adduct, pentafluorobenzenesulfonyl and vinyl sullen) which are capable of reacting with amino groups

Thus, in an interesting embodiment of the present invention the method further comprises the subsequent step (step d)) of converting an amino reactive functionality of the solid surface coated with the thus activated polyhydroxypolymer to another functionality (e.g. selected from carboxylic acid, amino, thiol, and maleimido) by reacting the amino reactive functionality with a reagent which comprises an amino group, preferably a primary amino group. The amino reactive functionalities may be functionalities selected from tresyl, tosyl, cyanogenbromide, triflyl, pentafluorobenzenesulphonyl and vinyl sulphone. The reagents for such an additional step are exemplified above and the conditions (e.g. in an aqueous buffered solution) will be known for the person skilled in the art.

Especially interesting are the cases within the above-mentioned embodiment

a) where the reagent which comprises an amino group has the general formula H_2N-C_nH_{2n}-COOH, where n is 1-20, preferably 1-8, whereby an immobilised carboxylic acid functionalised polyhydroxypolymer is formed,
b) where the reagent which comprises an amino group has the general formula H_2N-C_nH_{2n}-S-S-C_nH_{2n}-NH_2, where n is 1-10, preferably 1-4, and where the intermediate

thus formed is subsequently reduced, whereby an immobilised thiol functionalised polyhydroxypolymer is formed, and

c) where the reagent which comprises an amino group has the general formula H_2N-C_nH_{2n}-NH_2, where n is 1-20, preferably 1-8, whereby an immobilised amino functionalised polyhydroxypolymer is formed.

As it is believed that the coated solid surfaces are novel in themselves, the present invention also relates to solid surfaces coated with activated polyhydroxypolymers. Such solid surface may advantageously be prepared according to the method of the present invention, but alternative methods may also apply.

The solid surfaces prepared according to the invention are particularly useful for immobilising molecules of various origins. Examples of a particularly interesting group of molecules is biomolecules such as amino acids, oligo- and polypeptides (a special example is PNA), proteins, immunoglobulins, haptens, enzymes, antibodies (monoclonal and polyclonal), antigenes, polysaccharides, oligo- and polynucleotides (nucleic acids such as RNA and DNA), micro-organisms, procaryotic cells, eucaryotic cells, etc. It has been experienced that tresyl activated polyhydroxypolymers are especially suitable for the immobilisation of relatively short peptides and nucleic acids such as peptides consisting of 1-50, or 1-30, amino acids and nucleic acids consisting of 1-30, or 1-20, nucleotides.

Thus, the present invention also provides solid surfaces coated with an activated polyhydroxypolymer as described herein, where one or more biomolecules have been immobilised to said polyhydroxypolymer via at least a fraction of the activation groups. The biomolecules are typically selected from amino acids, oligo- and polypeptides, proteins, immunoglobulins, haptens, enzymes, antibodies, antigenes, polysaccharides, oligo- and polynucleotides, micro-organisms, procaryotic cells, eucaryotic cells. In a particularly interesting embodiment, the polyhydroxypolymer is a polysaccharide (in particular dextran) and the biomolecule is selected from peptides (including PNA) consisting of 1-30 amino acids and nucleic acids consisting of 1-20 nucleotides. The tresyl group as activation group on the polyhydroxypolymer is especially relevant in these instances.

The conditions for immobilisation of such (bio)molecule are known, see e.g. Hermanson, Mallia and Smith, Immobilized Affinity Ligand Techniques, Academic Press, 1992, Immo-

bilized Enzymes and Cells, in Methods in Enzymology, Vol 135, Mosbach, Ed., Academic Press, 1987, and US 5,516,673.

Thus, the present invention also relates to the use the solid surfaces obtained or obtainable according to the method of the present invention for immobilisation of biomolecules.

In another very interesting embodiment of the method according to the present invention, the resulting solid surface carry two types of activated polyhydroxypolymer so as to be able to immobilise a broader range of biomolecules. Alternatively, the polyhydroxypolymer may carry more than one type of functional groups thereby acting as a di-activated polyhydroxypolymer. This can be accomplished either by using two different activated polyhydroxypolymers in the contacting step or by only partial conversion of the functional groups of the polyhydroxypolymer already coated onto the solid surface.

The invention is further illustrated by the following examples:

EXAMPLES

If nothing else is noted chemicals were of analytical grade from Riedel de-Häen, Seelze, Germany.

A general method to test the peptide binding capacity

The peptide biotin-MP9 (biotin-FAQKEPAFLKEYHLL) was dissolved (0.01 mg/ml) in 0.1 M carbonate buffer, pH 9.6, and 100 µl was added to the wells to be tested. After 60 min incubation the wells were washed with washing buffer (PBS including 0.5 M NaCl and 1% Triton x-100 (Sigma)). Residual binding sites were blocked using carbonate buffer including 1% bovine serum albumin (BSA, Sigma), 15% polyethylene glycol (PEG) 8000 (Sigma), and 10 mM ethanol amine. After washing, the immobilized peptide was detected via the biotin group using a streptavidin-horse radish peroxidase (streptavidin-HRP, Amersham) conjugate in diluting buffer (washing buffer including 1% BSA) and o-phenylenediamine dihydrochloride (OPD, Sigma), 1 mg/ml in substrate buffer (citrate phosphate buffer, pH 5.0) as the chromogenic substrate.

Example 1. Synthesis of tresyl activated dextran (TAD)

Dextran (Sigma, mW 70000, freeze dried from water to remove water bound to the dextran), 4.5 g was dissolved in dry N-methyl-pyrrolidinone (NMP, 225 ml) at 90-92°C with magnetic stirring. After cooling to 40°C, 2,2,2-trifluoroethanesulfonyl chloride (tresyl chloride), 2764 µl, was added. After 15 min 2020 µl dry pyridine was added and the heating was removed. After 60 min stirring at room temperature (RT) the TAD was precipitated in 1200 ml cold ethanol. The precipitate was dissolved in 200 ml 0.5% acetic acid and dialyzed against 3 times 5 l 0.5% acetic acid in a dialysis membrane with a molecular cut off on 12000-14000 Da. After dialysis the TAD was freeze dried.

Example 2. Synthesis of tosyl activated dextran (TosAD)

Dextran (Sigma, mW 70000, freeze dried from water to remove water bound to the dextran), 0.8 g was dissolved in dry N-methyl-pyrrolidinone (NMP, 40 ml) at 90-92°C with magnetic stirring. After cooling to 60°C *p*-toluenesulfonyl (tosyl) chloride, 2.8 g dissolved

in dry NMP was added. After 1min 2 ml dry pyridine was added. After 60 min the precipitate is harvested by decanting the supernatant and precipitate is washed using 10 ml NMP and subsequently using 10 ml ethanol (99.9%). The precipitate is dissolved in 5 ml water and precipitated using 30 ml ethanol (99.9%). Subsequently the precipitate was dissolved in 5 ml water and freeze dried. The introduction of tosyl to the dextran could be detected by UV at 280 nm.

Example 3. Synthesis of maleimido activated dextran (MAD)

Dextran (Sigma, mW 70000, freeze dried from water to remove water bound to the dextran) 100 mg, was dissolved in dry 1-methyl-2-pyrrolidinone (5 ml) at 90-92°C with magnetic stirring. After cooling to room temperature *p*-maleimidophenyl isocyanate (PMPI, Bioaffinity Systems, Roscoe, Il, USA), 50 mg dissolved in 1 ml dry dimethyl sulfoxide, was added. After over night incubation the product was precipitated with 20 ml ethanol (99.9%), dissolved in 5 ml water and freeze dried.

Example 4. The effect of pH on coating TAD onto a solid phase

The effect of pH on the direct coating of activated polyhydroxypolymers, such as TAD, was examined by dissolving TAD in different buffers covering the pH range from 2.6 to 9.6. TAD was dissolved in 0.5% acetic acid (pH 2.6), citrate/phosphate buffer (0.035 M citrate, 0.075 M phosphate, pH 5.0), PBS (0.01 M phosphate, 0.15 M NaCl, pH 7.2) or carbonate buffer (0.1 M carbonate, pH 9.6) to a final concentration of 0.5 mg TAD/ml buffer. The solutions were dispensed into the wells of polystyrene microtitre plates (Polysorp, Nunc, Denmark), 150 µl/well, and incubated overnight at room temperature. The wells were then washed twice using 0.5% acetic acid and dried overnight at 37°C. The plates were tested as described in "general method to test the peptide binding capacity" and the result is shown in Fig 1. Coating at pH 2.6 and 5.0 resulted surfaces with the highest peptide binding capacities. Coating at pH 7.2 reduced the signal from the peptide binding by approx. 15%. Coating at pH 9.6 resulted in a signal reduction of approx. 70%. These results demonstrate that coating TAD directly on solid phases preferably should take place under acetic conditions, e.g. in 0.5% acetic acid or alternatively under neutral conditions.

Example 5. Coating TAD on solid phases composed of different materials

TAD, 0.5 mg/ml in 0.5% acetic acid, was added to the surface of different materials such as a nickel spatula, a glass test tube, a polycarbonate microtitre plate and a polystyrene microtitre plate and incubated over night at RT. After washing using 0.5% acetic acid and drying over night at 37°C the TAD coated materials were tested as described in "A general method to test the peptide binding capacity". The peptide binding test was performed on TAD coated as well as on not coated samples of each material and the results is shown in Fig 4. It is obvious the TAD coating makes the materials tested in this experiment capable of binding much more peptide than the uncoated materials.

Example 6. Coating with maleimido activated dextran (MAD) and identification of the chemical specificity of the MAD coated surface

Binding of cysteine to a microtitre plate coated with different amounts of MAD. MAD was added in serial dilutions, starting at 1 mg/ml, to a microtitre plate (MAXISorp) in 4 M NaCl in water and incubated 3 hours at RT. After washing with water the plate was dried over night at 37°C. Cysteine and lysine was added, 0.01 mg/ml in PBS, pH 7.2. After 1 hour incubation the plate was washed and biotin-NHS was added, 0.05 mg/ml in PBS including 0.1% Tween 20, pH 7.2. After 1 hour incubation at RT the wells were washed with washing buffer and streptavidin-HRP in diluting buffer (washing buffer including 1% BSA, pH 7.2), 1:1000, was added. Finally OPD, 1 mg/ml in substrate buffer was added. The reaction was stopped using 2 N H_2SO_4 the wells were read at 490 nm in an MRX ELISA reader (Dynex Technologies). Fig 6 shows the result and it is clear that the binding of cysteine is dependent on the precoating of the surface with MAD. Furthermore, this experiment demonstrates that cysteine (circles) binding takes place via the thiol group, as no binding was detected when lysine (triangles) was used. The specificity was also examined in a similar experiment using cysteine, lysine, glutamic acid and glycine (Fig 7). The amino acids were added to the MAD coated microtitre plate in PBS, 0.01 mg/ml, pH 7.2, and incubated 1 hour at RT. The detection of immobilized amino acids were the preformed using biotin-NHS as described above.

Example 7. The affect of high salt ionic strength when coating MAD on a microtitre plate

MAD was diluted from a stock solution (20 mg/ml in water) to 0.5 mg/ml in solutions of NaCl ranging from 0 to 4 M NaCl and added to a microtitre plate (MAXISorp). After 3

hours incubation the plates were washed with water and dried over night at 37°C. Cysteine and lysine, 0.01 mg/ml, (or buffer alone as control) were added dissolved in PBS and incubated 1 hour at RT. After washing, immobilization of cysteine or lysine was detected using biotin-NHS as described in example 6. The results are shown in Fig 5 and indicate that increased ionic strength when coating MAD results in increased binding of a thiol containing molecule, exemplified in this experiment by cysteine.

Example 8. Testing the amino acid side chain specificity of a TAD coated surface

An array of amino acids all with a t-butoxycarbonyl (Boc) on the α-amino group were added to the wells of a TAD coated polystyrene microtitre plate and incubated for 1 hour at RT. After washing the wells with water, the Boc group was stripped off the immobilized amino acids by treatment with 95% trifluoroacetic acid (TFA) in water (black bars in Fig 2), or treated with water only for control (white bars in Fig 2) 30 min at RT. The wells were washed with water and biotin-N-hydroxysuccinimide (biotin-NHS) was added, 0.05 mg/ml in PBS including 0.1% Tween 20, pH 7.2. After 1 hour incubation at RT the wells were washed with washing buffer and streptavidin-HRP in diluting buffer (washing buffer including 1% BSA, pH 7.2), 1:1000, was added. Finally OPD, 1 mg/ml in substrate buffer was added. The reaction was stopped using 2 N H_2SO_4 the wells were read at 490 nm in an MRX ELISA reader (Dynex Technologies). From Fig 2 it is obvious the TAD coated surface has a preference for lysine and cysteine side chains, i.e. primary amine and thiol. This is in accordance with the theory.

Example 9. Identification of B-cell epitopes using peptides covalently bound to a TAD coated microtitre plate

Peptides covering then entire sequence of murine tumor necrosis factor α (mTNFa) were synthesised as 15-mer peptides with a 5 amino acid overlap. These peptides were immobilised (0.05 mg/ml in carbonate buffer, 2 hours at RT) on a TAD coated microtitre plate and on a conventional microtitre plate of the high binder type (MAXISorp). Subsequently, after washing and blocking (as described in the "A general method to test the peptide binding capacity"), anti-TNFα anti-serum was added. Peptides containing B-cell epitopes were then expected to be recognised by the anti-serum. Fig 3 shows the result of this B-cell epitope identification assay. More of the covalently immobilised peptides (A) were recognised than of the non-covalently immobilised peptides (B). Thus, if the experiment

had been performed on the conventional plate only and not on the TAD coated plate, information about B-cell epitopes in the N-terminal part of the TNFα molecule would not have been obtained. The part of the experiment performed on the conventional plate (B) did not give any information that could not be obtained from the TAD coated plate (A).

Example 10. Generation of a surface with functional carboxylic groups using a TAD coated surface as platform

To a TAD coated microtitre plate 6-amino hexanoic acid (Sigma), 1 mg/ml in carbonate buffer, was added and incubated 2 hours at RT. The plate was then washed and the presence of functional carboxylic groups on the surface was tested by activation of the carboxylic groups and subsequent binding of a labelled peptide. The carboxylic groups were activated by adding fresh solutions of 1-(3-diaminopropyl)-3-ethylcarbodiimide (EDC, 0.18 mg/ml in water) and N-hydroxy succinimide (NHS 1.23 mg/ml in water). As control, EDC and NHS were either added together, alone or only water was added and incubated for 30 min at RT. After washing with water the biotinylated peptide (biotin-FAQKEPAFLKEYHLL) was added, 0.01 mg/ml, in PBS including 0.2% Tween 20. The result is seen in Fig 8. Clearly, only when both EDC and NHS was used for activation a proper binding of the peptide was seen. EDC alone also generates a reactive ester with a carboxylic group but it is very unstable in water. NHS alone cannot form a reactive product but if EDC and NHS is present at the same time, the EDC ester reacts rapidly with NHS and forms a stable NHS ester.

Example 11. Generation of a surface with functional thiol groups using a TAD coated surface as platform

To a TAD coated microtitre plate 2,2'-dithio-bis(ethylamine) (cystamine, Sigma), 1 mg/ml in carbonate buffer, was added and incubated 2 hours at RT. After washing with water, sodium dithionite (Sigma), 2 mg/ml in water, was added and incubated for 3 hours at RT. The plate was washed with water and the generated thiol groups were detected by adding biotin-maleimide (Sigma, 0.05 mg/ml in PBS) which specifically reacts with thiol groups, and incubate 1 hour at RT. To test for residual amine binding capacity (residual tresyl groups) the peptide biotin-MP9 (biotin- FAQKEPAFLKEYHLL) was added, 0.01 mg/ml in carbonate buffer, pH 9.6. The immobilized biotin groups (from either biotin-MP9 or biotin-maleimide) were detected using a streptavidin-horse radish peroxidase (strept-

avidin-HRP, Amersham) conjugate in diluting buffer (washing buffer including 1% BSA) and o-phenylenediamine dihydrochloride (OPD, Sigma), 1 mg/ml in substrate buffer (citrate phosphate buffer, pH 5.0) as the chromogenic substrate. Fig 9 shows how the TAD coated surface bound the biotin-maleimide (TAD/biotin-mal) or the peptide biotin-MP9 (TAD/biotin-MP9) in the two first bars. The third bar is binding of the maleimide group to the thiol enriched surface (Thio/biotin-mal) and the last bar is binding of biotin-MP9 (Thio/biotin-MP9) to the thiol enriched surface. It is obvious the TAD coated surface by treatment with cystamine and sodium dithionite lost its ability to bind the peptide biotin-MP9 but gained the ability to bind a thiol specific reagent as biotin-maleimide.

CLAIMS

1. A method for coating a solid surface with a water-soluble activated polyhydroxypolymer, where the solid surface comprises substantially no amino groups, imino groups or thiol groups, the method comprising the step of:

a) contacting a coating solution of the activated polyhydroxypolymer in an aqueous medium having a pH in the range of 1.5-10 and/or having an ion strength in the range of 0.1 to 8 with the solid surface so as to obtain a bonding of the activated polyhydroxypolymer to the solid surface;
b) rinsing the solid surface having the activated polyhydroxypolymer attached thereto with a rinse solution; and
c) optionally drying the solid surface having the activated polyhydroxypolymer attached thereto.

2. A method according to claim 1, wherein the activated polyhydroxypolymer comprises substantially no amino, ammonium, thiol, phenyl, naphthyl, pyridyl or pyridone groups.

3. A method according to any of the preceding claims, wherein the polyhydroxypolymer is selected from naturally occurring polyhydroxy compounds and synthetic polyhydroxy compounds.

4. A method according to claim 3, wherein the polyhydroxypolymer is a polysaccharide.

5. A method according to claim 4, wherein the polysaccharide is selected from dextran, cellulose, agarose and starch, preferably dextran.

6. A method according to claim 3, wherein the polyhydroxypolymer is selected from polyvinylalcohol, poly(hydroxymethylmethacrylate), poly(hydroxyethylmethacrylate), poly(hydroxypropylmethacrylate), and the corresponding copolymers.

7. A method according to claim 6, wherein the polyhydroxypolymer is polyvinylalcohol.

8. A method according to any of the preceding claims, wherein the (weight) average molecular weight of the polyhydroxypolymer before activation is at least 1,000, such as at least 2,000, preferably in the range of 2,500-2,000,000, more preferably in the range of 3,000-1,000,000, in particular in the range of 5,000-500,000.

9. A method according to any of the preceding claims, wherein the polyhydroxypolymer is water soluble to an extent of at least 10 mg/ml, preferably at least 25 mg/ml, such as at least 50 mg/ml, in particular at least 100 mg/ml, such as at least 150 mg/ml.

10. A method according to any of the preceding claims, wherein the ratio between C (carbon atoms) and OH groups (hydroxy groups) in the polyhydroxypolymers before activation is in the range of 1.3 to 2.5, such as in the range of 1.5-2.3, preferably in the range of 1.6-2.1, in particular in the range of 1.85-2.05.

11. A method according to any of the preceding claims, wherein the polyhydroxypolymer is activated with functional groups selected from tresyl (trifluoroethylsulphonyl), maleimido, cyanogenbromide, tosyl (p-toluenesulfonyl), triflyl (trifluoromethanesulfonyl), pentafluorobenzenesulfonyl, and vinyl sulphone groups, preferably selected from tresyl, maleimido, tosyl, triflyl, pentafluorobenzenesulfonyl, and vinylsulphone groups, in particular selected from tresyl, maleimido, and tosyl groups.

12. A method according to any of the preceding claims, wherein the functional groups of the activated polyhydroxypolymers are attached to the polyhydroxypolymer via a fraction of the hydroxy groups of the polyhydroxypolymer.

13. A method according to any of the preceding claims, wherein the skeleton of the polyhydroxypolymer is substantially unaffected by the activation.

14. A method according to any of the preceding claims, wherein substantially no aldehyde groups are included in the polyhydroxypolymer.

15. A method according to any of the preceding claims, wherein the activated polyhydroxypolymer is tresyl activated dextran.

16. A method according to any of the claims 1-14, wherein the activated polyhydroxy-polymer is maleimido activated dextran.

17. A method according to any of the claims 1-14, wherein the activated polyhydroxy-polymer is tosyl activated dextran.

18. A method according to any of the preceding claims, wherein the solid surface is the surface of a body of organic polymers, glasses, ceramics or metals.

19. A method according to claim 18, wherein the organic polymer is selected from polystyrene, polycarbonate, polypropylene, polyethylene, polyethyleneglycol terephthalate, polyvinylacetate, polymethylpentene, polyvinylpyrrolidinone, polyacrylonitrile, polymethylmethacrylate, and polyvinylchloride, preferably from polystyrene and polycarbonate.

20. A method according to claim 18, wherein the glass is selected from borosilicate glass and soda-lime glass.

21. A method according to claim 18, wherein the metal is selected from nickel, iron, copper gold, silver, aluminium, and zinc.

22. A method according to any of the claims 1-18, wherein the solid surface is the surface of a polystyrene body, a polycarbonate body, a borosilicate glass body, or a soda-lime glass body.

23. A method according to any of the claims 18-22, wherein the body is in the form of a sheet, a film, a bead, a pellet, a disc, a plate, a ring, a rod, a net, a filter, a tray, a microtitre plate, a stick, or a multi-bladed stick, preferably a microtitre plate, a stick or a bead.

24. A method according to any of the preceding claims, wherein the solid surface is selected from the surface of a polystyrene microtitre plates, the surface of a polystyrene bead, the surface of a polystyrene stick, the surface of a polycarbonate microtitre plate, the surface of a glass bead, and the surface of a glass plate.

25. A method according to any of the preceding claims, wherein the coating solution used in step a), apart from the polyhydroxypolymers, comprises a pH adjusting agent and/or a chaotropic agent, and optionally one or more auxiliary components.

26. A method according to any of the preceding claims, wherein the concentration of activated polyhydroxypolymer in the coating solution is in the range of 0.001 mg/ml to 5 mg/ml, typically in the range of 0.01 mg/ml to 1 mg/ml, and preferably in the range of 0.1 mg/ml to 0.5 mg/ml.

27. A method according to any of the preceding claims, wherein the amount of activated polyhydroxypolymer used per area unit of the surface to be coated is in the range of 0.01-500 $\mu g/cm^2$, preferably in the range of 0.06-200 $\mu g/cm^2$, in particular in the range of 0.1-50 $\mu g/cm^2$.

28. A method according to any of the preceding claims, wherein pH of the coating solution is in the range of 1.5-10, preferably in the range of 2.0-7.5, more preferably in the range of 2.0-5.5.

29. A method according to any of the preceding claims, wherein the ionic strength of the coating solution comprising the polyhydroxypolymer is in the range of 0.1-8, preferably in the range of 0.5-6, more preferably in the range of 0.8-5, in particular in the range of 1.2-4.

30. A method according to any of the preceding claims, wherein the drying in step d) is performed at a temperature in the range of 20°C-56°C, preferably in the range of 20°C-45°C.

31. A method according to any of the preceding claims, wherein the stability of the coated surface is so that the difference in absorbance for the most absorbing amino acid in a test for the amino acid side chain specificity (as described for TAD in example 6) when tested on an uncoated solid surface and on a similar solid surface coated with the activated polyhydroxypolymer in question has decreased with at the most 25%, preferably at the most 15%, more preferably a the most 10%, in particular at the most 5%, after storage at 37°C for one year.

32. A method according to any of the preceding claims, wherein the method comprises

a) contacting a solution of a tresyl activated polysaccharide in an aqueous medium having a pH in the range of 1.5-7.5 with a polystyrene surface;

b) rinsing the polystyrene surface with a rinse solution; and

c) drying the polystyrene surface coated with the tresyl activated polysaccharide.

33. A method according to any of the claim 1-31, wherein the method comprises

a) contacting a solution of a maleimido activated polysaccharide in an aqueous medium having a ionic strength in the range of 0.5-6 with a polystyrene surface;

b) rinsing the polystyrene surface with a rinse solution; and

c) drying the polystyrene surface coated with the maleimido activated polysaccharide.

34. A method according to any of the claim 1-31, wherein the method comprises

a) contacting a solution of a tosyl activated polysaccharide in an aqueous medium having a pH in the range of 1.5-7.5 with a polystyrene surface;

b) rinsing the polystyrene surface with a rinse solution; and

c) drying the polystyrene surface coated with the tosyl activated polysaccharide.

35. A method according to any of the preceding claims, comprising the further subsequent step of

d) converting an amino reactive functionality of the solid surface coated with the activated polyhydroxypolymer to another functionality by reacting the amino reactive functionality with a reagent which comprises an amino group.

36. A method according to claim 35, wherein the reagent which comprises an amino group has the general formula $H_2N\text{-}C_nH_{2n}\text{-}COOH$, where n is 1-20, preferably 1-8, whereby an immobilised carboxylic acid functionalised polyhydroxypolymer is formed

37. A method according to claim 35, wherein the reagent which comprises an amino group has the general formula $H_2N\text{-}C_nH_{2n}\text{-}S\text{-}S\text{-}C_nH_{2n}\text{-}NH_2$, where n is 1-10, preferably 1-4, and where the intermediate thus formed is subsequently reduced, whereby an immobilised thiol functionalised polyhydroxypolymer is formed.

38. A method according to claim 35, wherein the reagent which comprises an amino group has the general formula $H_2N\text{-}C_nH_{2n}\text{-}NH_2$, where n is 1-20, preferably 1-8, whereby an immobilised amino functionalised polyhydroxypolymer is formed.

39. A method according to claim 38, wherein the immobilised amino functionalised polyhydroxypolymer is reacted with a maleimide reagent, whereby an immobilised maleimido functionalised polyhydroxypolymer is formed.

40. A method according to any of claims 35-39, wherein only a fraction of the amino reactive functionalities are reacted, whereby two different types of functionalities are included in the immobilised polyhydroxypolymer.

41. A method according to any of the preceding claim wherein step c) is omitted.

42. A method according to any of the preceding claim wherein step c) is included.

43. A solid surface coated with an activated polyhydroxypolymer obtainable by the method defined in any of the claims 1-42.

44. A solid surface coated with an activated polyhydroxypolymer obtainable by the method defined in any of the claims 1-42, where one or more biomolecules have been immobilised to said polyhydroxypolymer via at least a fraction of the activation groups.

45. A solid surface according to claim 44, wherein the biomolecules are selected from amino acids, oligo- and polypeptides, proteins, immunoglobulins, haptens, enzymes, antibodies, antigenes, polysaccharides, oligo- and polynucleotides, micro-organisms, procaryotic cells, eucaryotic cells.

46. A solid surface according to claim 44 or 45, wherein the polyhydroxypolymer is a polysaccharide, and the biomolecule is selected from peptides consisting of 1-30 amino acids and nucleic acids consisting of 1-20 nucleotides.

47. A solid surface according to any of the claims 44-46, wherein the activated polyhydroxypolymer is activated with tresyl groups.

48. The use of a solid surface coated with an activated polyhydroxypolymer obtainable by the method defined in any of the claims 1-42 in the immobilisation of biomolecules.

Fig. 1

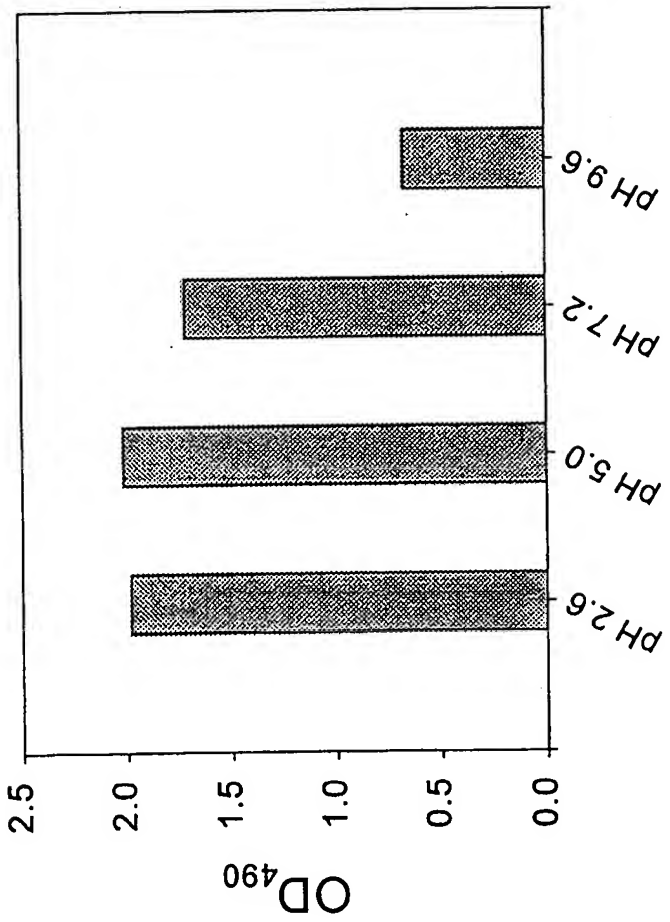
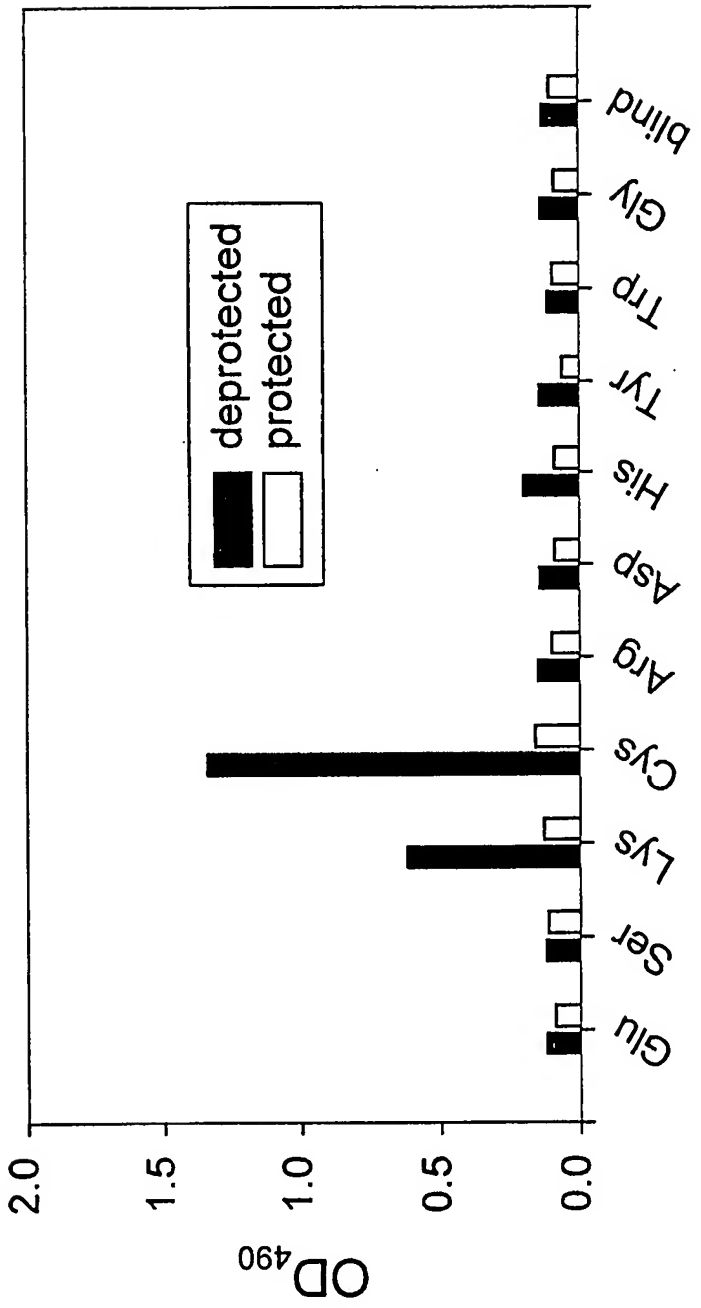


Fig. 2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protected
blind
Gly
Trp
Tyr
His
Asp
Arg
Cys
Lys
Ser
Glu
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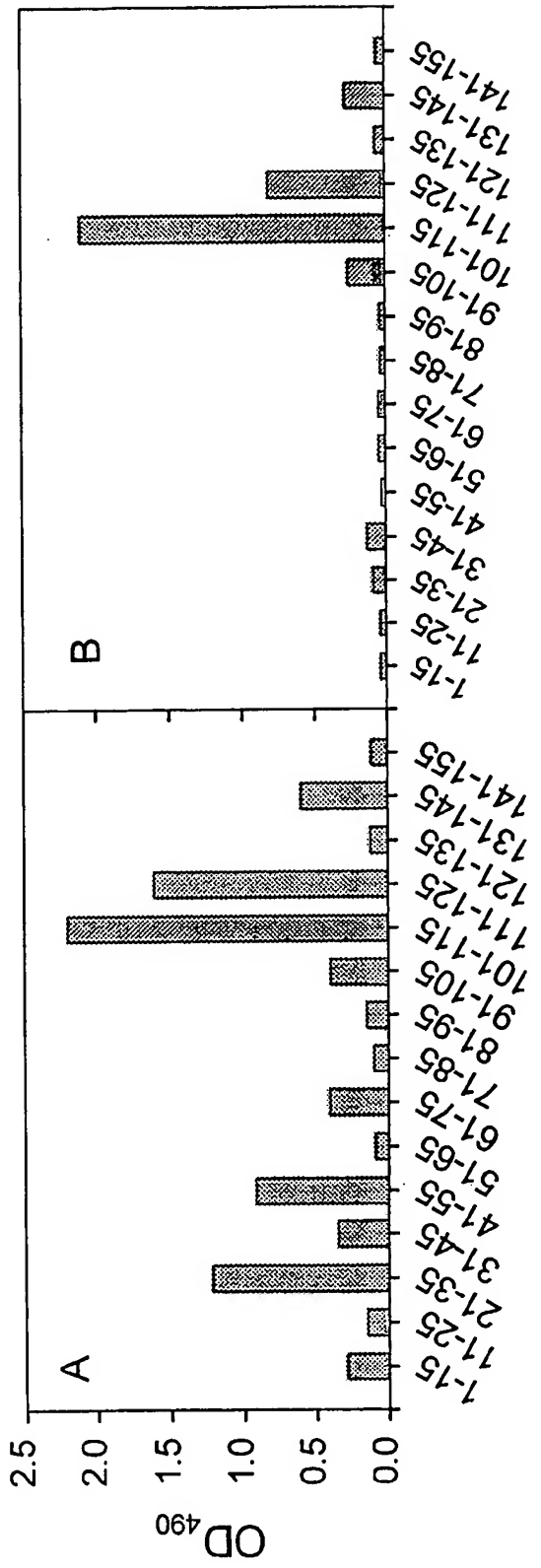
0.5
1.0
1.5
2.0
OD490

Fig. 3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2.0
1.5
1.0
0.5
0.0
A
B
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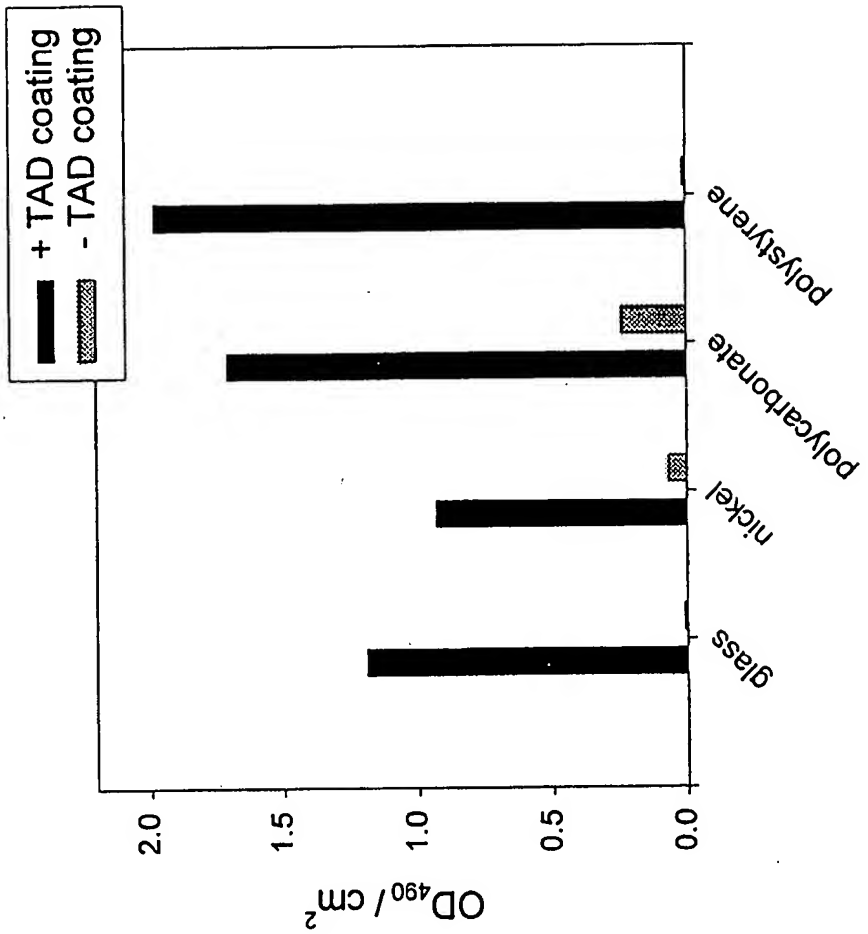
11-25
21-35
31-45
41-55
51-65
61-75
71-85
81-95
91-105
101-115
111-125
121-135
131-145
141-155

Fig. 4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- TAD coating
polystyrene
polycarbonate
nickel
glass
0.0
0.5
1.0
1.5
2.0
OD490 / cm2

Fig. 5

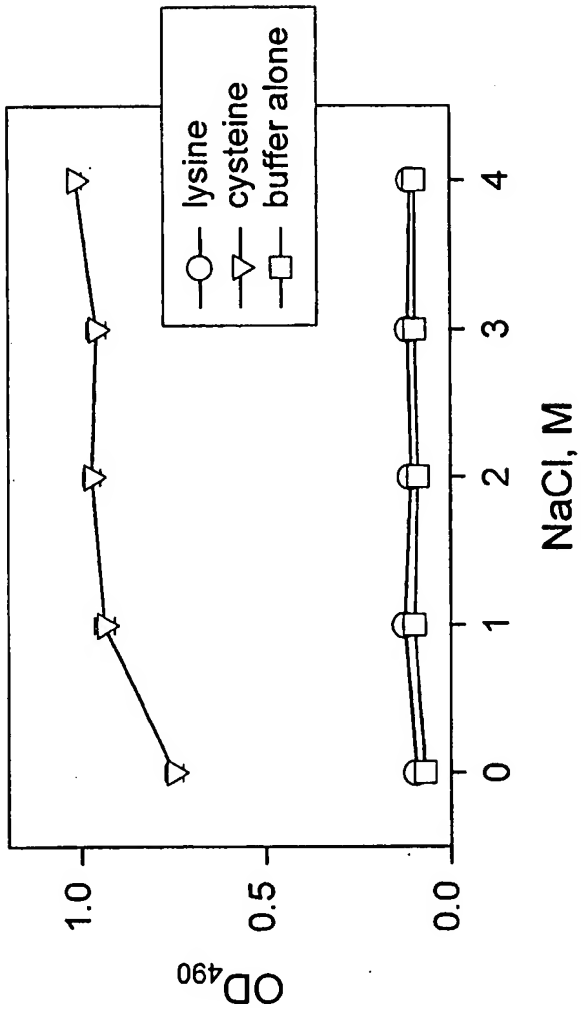


Fig. 6

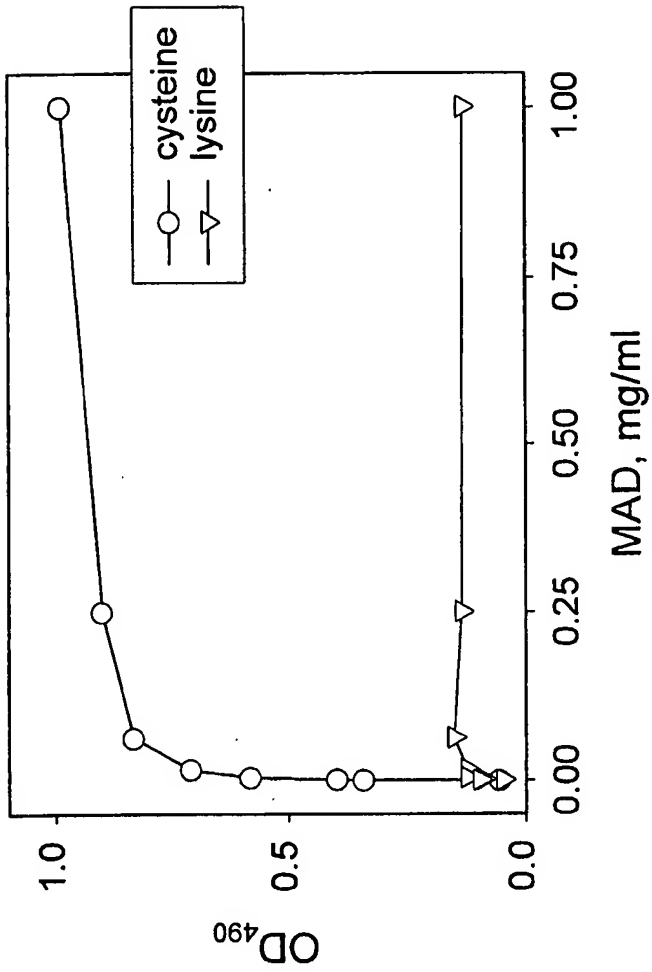


Fig. 7

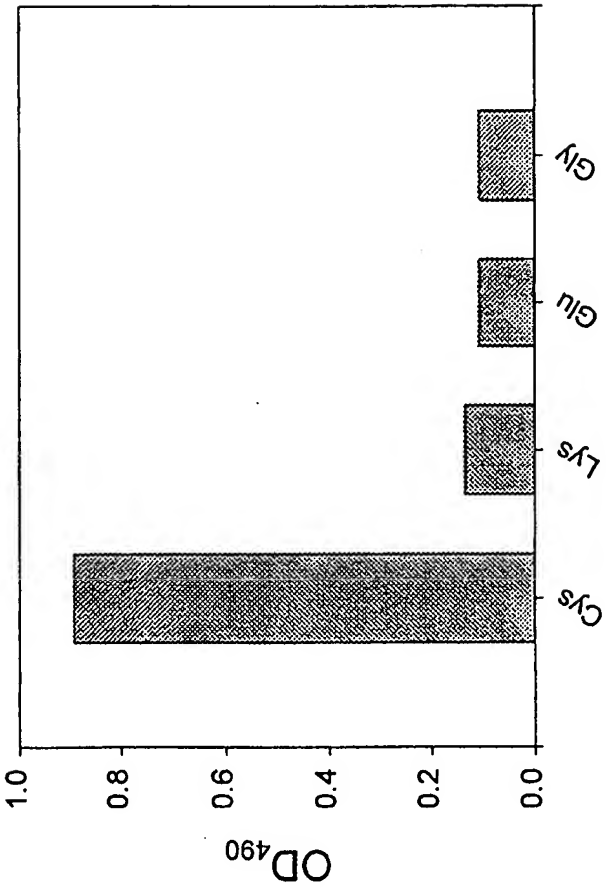


Fig. 8

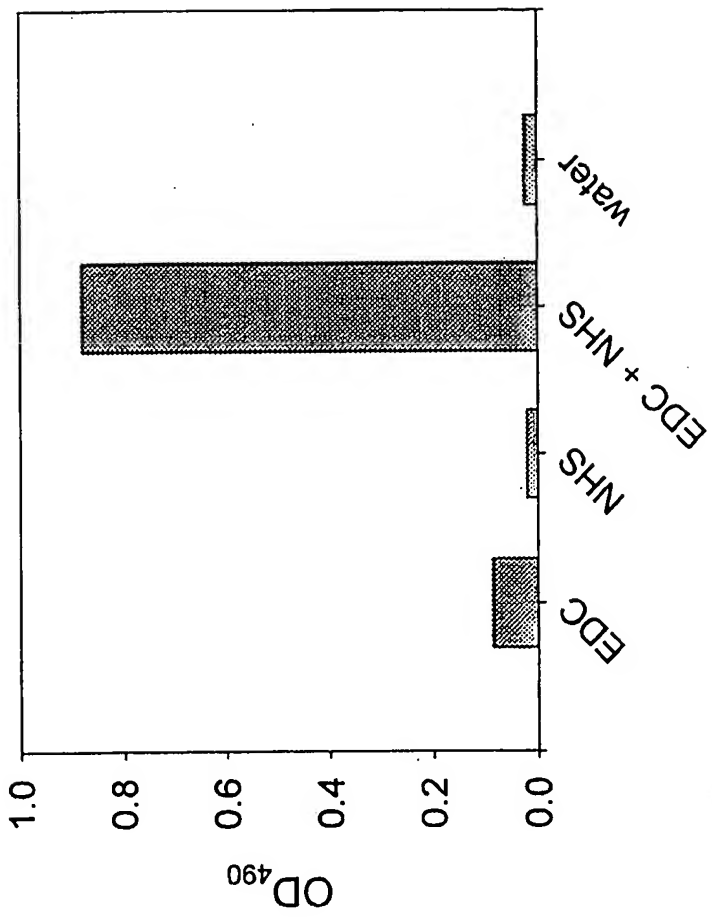
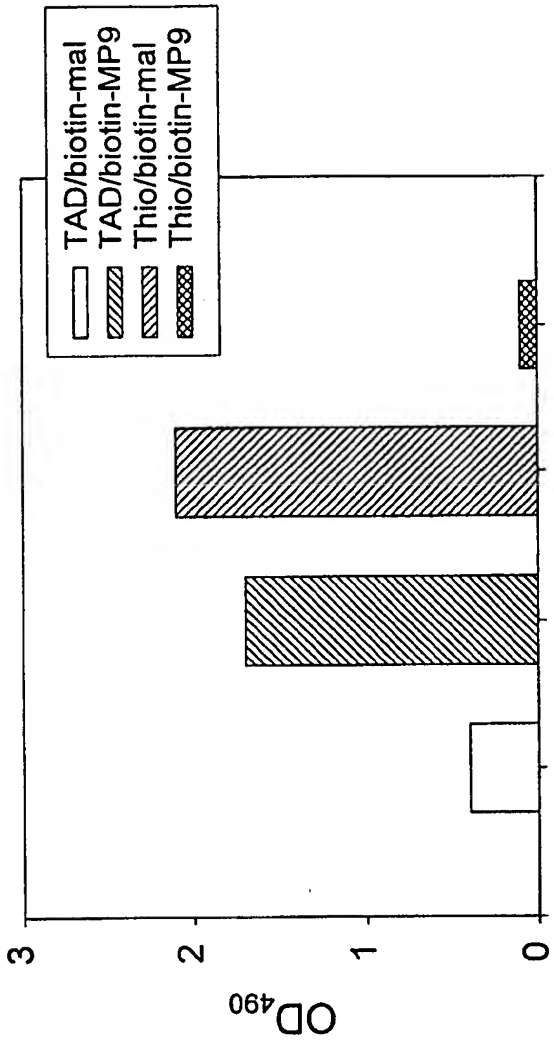


Fig. 9


TAD/biotin-mal
TAD/biotin-MP9
Thio/biotin-mal
Thio/biotin-MP9
OD490
0
1
2
3

INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 99/00407

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C09D201/08 C08J7/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C09D C08J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 91 09877 A (BEROL NOBEL AB) 11 July 1991 (1991-07-11) cited in the application claims 1,4,5,7 page 5, line 18 - line 30 example 1	1
A	US 5 281 660 A (BRYHAN MARIE D ET AL) 25 January 1994 (1994-01-25) cited in the application claim 1	1
A	US 5 275 838 A (MERRILL EDWARD W) 4 January 1994 (1994-01-04) claim 1	1

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 1999	01/12/1999

Name and mailing address of the ISA	Authorized officer
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1

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/DK 99/00407

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	DE 43 31 925 A (WINDI WINDERLICH GMBH) 16 March 1995 (1995-03-16) claims 1,11,17 example -----	1

1

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Information on patent family members

International Application No
PCT/DK 99/00407

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